PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (12B Supplementary) 4237-4241 Dec. 2024

In-Vitro Anthelminthic Potential of Ethanolic and Methanolic Stem Bark Extract of *Adansonia digitata* Linn (Baobab Tree) on earthworms (*Pheritima posthuma*)

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ABSTRACT: Medicinal plants have been used for decades by rural dwellers to alleviate suffering due to parasitic diseases and interest have grown in recent times on their application as alternatives for deworming of animals. Hence, this study was undertaken to investigated the *in vitro* anthelminthic potential of ethanolic and methanolic stem bark extracts of activity of *Adansonia digitata* Linn (Baobab Tree) on earthworms (*Pheritima posthuma*) using 12.5, 25.0, 50.0 and 100.0 mg/mL ethanolic and methanolic extract concentrations. Data obtained indicates that after 60-minute of exposure, the mortality pattern mirrored that observed at 30 minutes, with the mean number of live worms decreasing from 1.25±1.26 (at 12.5 mg/ml) to 0±0 (at 100 mg/ml) for the ethanol extract and 1.5±0.57 (at 12.5 mg/ml) to 0±0 (at 100 mg/ml) for the methanol extract. This reduction was statistically significant ($p<0.05$) and compared favourably with Albendazole (40 mg/ml), which is the positive control. A dose dependent inverse relationship was observed with increase in the number of dead worms and decrease in the number of earthworm alive. After 30 minutes of exposure, the number of dead earthworms rose to a 100% at 100mg/mL concentration for both extracts. Overall, the anthelmintic activity was significantly greater after 60 minutes compared to 30 minutes of exposure, indicating enhanced effectiveness over time across the treatment groups.

DOI: https://dx.doi.org/10.4314/jasem.v28i12.36

Cite this Article as: BIU, A. A; WASAGWA, J; ONYICHE, T. E; MUHAMMAD, F. M. (2024). *In-Vitro* Anthelminthic Potential of Ethanolic and Methanolic Stem Bark Extract of *Adansonia digitata* Linn (Baobab Tree) on earthworms (*Pheritima posthuma*). *J. Appl. Sci. Environ. Manage.* 28 (12B Supplementary) 4237-4241

Dates: Received: 22 October 2024; Revised: 20 November 2024; Accepted: 08 December 2024; Published: 31 December 2024

Keywords: Medicinal plants; Worms; Folkloric; Helminths; Solvent

Adansonia digitata commonly referred to as "Baobab", is an indigenous plant, which is native in the sub-Saharan regions of Africa, including Burkina Faso, Senegal, Sudan, Mali, Mozambique, Kenya, and Nigeria (Kamanula, 2018). This plant, *Adansonia digitata* L. (*Bombacaceae*) is an integral component of the diet and traditional medicine of African communities for centuries (Braca *et al*., 2018; Makawi *et al*., 2019). Various parts of the Baobab tree, including the seeds, leaves, fruit, and cortex, have been utilized in the treatment of a wide range of malady including fever, malaria, cough, diarrhea, dysentery, hemoptysis, tuberculosis, microbial infections, and parasitic worms (Li *et al*., 2017). Additionally, the oil and seeds are widely used for the management of muscle wounds, dandruff, and other skin conditions (Zahid *et al*., 2017). The folkloric uses has been attributed to the plant antioxidant, immune-stimulant, analgesic, antimicrobial, antiviral, anticarcinogenic,

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antidepressant, cardioprotective, hepatoprotective, antidiabetic, and anti-inflammatory properties (Suliman *et al*., 2020; Cicolari *et al*., 2020). The chemical composition of the various components of the Baobab tree have been analyzed in numerous studies (Chadare *et al*., 2017; Ismail *et al*., 2019; Tsetegho Sokeng *et al*., 2019). The anatomical and physiological resemblance of earthworms with parasitic gastrointestinal nematodes in humans and animals makes them a suitable model for anthelminthic studies (Nirmal *et al*., 2007; Ashok, 2010). Hence, the objective of this paper was to investigate the in vitro anthelminthic potential of ethanolic and methanolic stem bark extracts of *Adansonia digitata* Linn (Baobab Tree) on earthworms (*Pheritima posthuma*)

MATERIALS AND METHODS

Collection and Authentification of Adansonia digitata L: The stem bark of *Adansonia digitata* L. (*Bombacaceae*) was collected from the surroundings near the University of Maiduguri Ramat Library in Borno State Nigeria. Botanical identification of the plant was done by botanist from the Department of Biological Sciences, University of Maiduguri and a voucher number CHM/21/0068 was allocated and the specimen deposited in the herbarium of the Faculty of Science, University of Maiduguri.

Preparation of Ethanol and Methanol Extracts of Adansonia digitata L. Stem Bark: Fresh stem bark peels of *Adansonia digitata* L. were air-dried at room temperature to prevent degradation or denaturation of their phytocompounds and then ground into a fine powder using pestle and mortar, weighed using a Precisa analytical balance (520 PT series) to obtain a 1000g . 500 g was mixed with solvents (80% methanol and 80% ethanol respectively) and subjected to a reflux extraction method for 4 h. Each mixture was filtered and the filtrate was concentrated on a rotary evaporator at 40 °C. Thereafter, using a water bath at 50 °C in a water, the concentrate was dried to obtain an extract each weighing 40g. The extract was stored in a refrigerator at 4 °C until used.

Collection of Earthworms: The earthworms (*Pheritima posthuma),* approximately 2.0 to 6.5 mm in length, were used for the study and collected from water-logged areas in the Agricultural farm of the University of Maiduguri, where moist humus soil is available. After collection, the earthworms were washed using water to ensure the removal of all faecal matter. The choice of the earthworm was due to its anatomical and physiological resemblance to parasitic gastrointestinal nematodes in humans and animals (Nirmal *et al*., 2007).

Anthelmintic Testing: The anthelmintic activity was evaluated using the standard protocol described by Ajaiyeoba *et al*. (2001). Five earthworms (*Pheritima posthuma*) were placed in each Petri dish. The *in vitro* assay used four different concentrations of the extracts: 12.5 mg/mL, 25.0 mg/mL, 50.0 mg/mL, and 100 mg/mL. For each concentration tested, it was done in four (4) replicates. After immersing the earthworms for 30 and 60 minutes, they were observed for signs of paralysis and death. Death was confirmed if the worm did not move or respond to stimuli when shaken vigorously and if the earthworm changed color to pale white. The number of dead and alive earthworms was recorded. Additionally, positive (Albendazole) and negative (physiological saline solution) control experiments were conducted, each in four replicates.

Data Analysis: Student's t-test and one-way analysis of variance was used in the analysis of the obtained data. Results were expressed as mean ± standard deviation with p-values equal to or less than 0.05 considered statistically significant (Joonlee and Joong-Bae, 2015)

RESULTS AND DISCUSSION

Table 1 displays the anthelmintic effects of the ethanol extract of *Adansonia digitata* stem bark on earthworms subjected to varying concentrations. A dose-dependent response was noted, with an increase in the number of dead worms and a decrease in the number of live worms. After 30 minutes of exposure, the number of dead earthworms rose to a 100% at 100mg/mL concentration. Following a 60-minute exposure, the mortality pattern mirrored that observed at 30 minutes, with the mean number of live worms decreasing from 1.25±1.26 (at 12.5 mg/ml) to 0±0 (at 100 mg/ml). This reduction was statistically significant ($p<0.05$) and compared favourably with the positive control of Albendazole (40 mg/ml). Overall, the anthelmintic activity was significantly greater after 60 minutes compared to 30 minutes of exposure, indicating enhanced effectiveness over time across the treatment groups. Table 2 presents the anthelmintic effects of the methanol extract of *Adansonia digitata* stem bark on earthworms exposed to various concentrations. A dose-dependent response was observed, with an increase in the number of dead worms corresponding to a decrease in the number of live worms. After 30 minutes of exposure, the number of dead earthworms rose to 20 (100%) at 100 mg/mL concentration. After 60 minutes, the mortality trend was similar, with the mean number of live worms decreasing from 1.5±0.57 (at 12.5 mg/ml) to 0±0 (at 100 mg/ml). This reduction in live worms was statistically significant ($p <0.05$) and compared favourably with Albendazole (40 mg/ml) positive control. Overall, the anthelmintic activity was notably greater after 60 minutes of exposure than after 30 minutes, demonstrating enhanced effectiveness over time across the various treatment groups.

Table 1: Anthelminthic effects of different concentrations of ethanol extract of *Adansonia digitata* stem bark on earthworms

Treatment Groups/ Extracts Concentration (mg/mL)	30 Minutes Post Immersion /Exposure				60 Minutes Post Immersion/Exposure			
	No/(%) of Alive	(Mean ± S.D) of Alive (range)	No/(%) of Dead	(Mean ± S.D) of Dead (range)	No/(%) of Alive	(Mean ± S.D) of Alive (range)	No/ (%) of Dead	(Mean ± S.D) of Dead (range)
12.5	17 (85.0)	$4.25±0.95^{a}$ (3 - 5)	3 (15.0)	$0.75±0.95^{a}$ (0 - 2)	5 (25.0)	$1.25±1.26^{a}$ (0 – 3)	15 (75.0)	$3.75±1.26^{a}$ (2 – 5)
25	15 (75.0)	$3.75±0.5^{a}$ (3 - 5)	5 (25.0)	$1.25±0.5^{a}$ (0 - 2)	10 (50.0)	$2.5±1.0^{a}$ (1 – 3)	10 (50.0)	$2.5±1.0^{a}$ (2 – 4)
50	11 (55.0)	$2.75±0.5^{a}$ (2 - 3)	9 (45.0)	$2.25±0.5^{a}$ (2 - 3)	5 (25)	$1.25±1.26^{a}$ (0 – 3)	15 (75.0)	$3.75±1.26^{a}$ (2 – 5)
100	0 (0)	$0±0^{bc}$	20 (100.0)	$5±0^{b}$	0 (0)	$0±0^{bc}$	20 (100.0)	$5±0^{b}$
Albendazole 40 mg/mL (Positive control)	0 (0)	$0±0^{bc}$	20 (100.0)	$5±0^{b}$	0 (0)	$0±0^{bc}$	20 (100.0)	$5±0^{b}$
Normal Saline (Negative control)	20 (100.0)	$5±0^{a}$	0 (0)	$0±0^{a}$	20 (100.0)	$5±0^{d}$	0 (0)	0±0

Mean ± SD values within columns with different superscripts are significantly (p< 0.05), N = 20 (Total number of earthworms exposed to each extract concentration

Table 2: Anthelminthic effects of different concentrations of methanol extract of *Adansonia digitata* stem bark on earthworms

Treatment Groups/ Extracts Concentration (mg/mL)	Post immersion /Exposure 30 minutes				Post immersion/Exposure 60 minutes			
	No/(%) of Alive	(Mean ± S.D) of Alive (range)	No/(%) of Dead	(Mean ± S.D) of Dead (range)	No/(%) of Alive	(Mean ± S.D) of Alive (range)	No/ (%)of Dead	(Mean ± S.D) of Dead (range)
12.5	17 (85.0)	$4.25±0.95^{a}$ (3 - 5)	3 (15.0)	$0.75±0.95^{a}$ (0 - 2)	6 (30.0)	$1.5±0.57^{a}$ (1 – 2)	14 (70.0)	$3.5±0.57^{a}$ (3 – 4)
25	16 (80.0)	$4.0±0.82^{a}$ (3 - 5)	4 (20.0)	$1.0±0.82^{a}$ (0 - 2)	3 (15.0)	$0.75±0.5^{a}$ (0 – 1)	17 (85.0)	$4.25±0.5^{a}$ (4 – 5)
50	13 (65.0)	$3.25±0.5^{a}$ (3 - 4)	7 (35.0)	$1.75±0.5^{a}$ (1 - 2)	7 (35)	$1.75±0.5^{a}$ (1 – 2)	13 (65.0)	$3.25±0.5^{a}$ (3 – 4)
100	0 (0)	$0±0^{bc}$	20 (100.0)	$5±0^{b}$	0 (0)	$0±0^{bc}$	20 (100.0)	$5±0^{b}$
Albendazole 40 mg/mL (positive control)	0 (0)	$0±0^{bc}$	20 (100.0)	$5±0^{b}$	0 (0)	$0±0^{bc}$	20 (100.0)	$5±0^{b}$
Normal Saline (Negative control)	20 (100.0)	$5±0^{a}$	0 (0)	$0±0^{a}$	20 (100.0)	$5±0^{d}$	0 (0)	0±0

Mean ± SD values within columns with different superscripts are significantly (p< 0.05), N = 20 (Total number of earthworms exposed to each extract concentration)

The widespread and indiscriminate use of anthelmintic drugs for controlling gastrointestinal nematodes (GIN) has led to a growing global issue of anthelmintic resistance (Chan-Pérez *et al*., 2016). In response, there is increasing interest in using plant-based nematicidal products as alternatives to chemical anthelmintics. This study presents promising findings regarding the ethanol and methanol extracts of *Adansonia digitata*. The *in vitro* tests demonstrated the potential anthelmintic activity of these extracts against earthworms, chosen due to their physiological similarity to gastrointestinal roundworms (Vigar, 1984). The efficacy of the extract increased in a dose- and time-dependent manner, with higher doses and longer exposure times (from 30 minutes to 60 minutes) resulting in greater mortality of the earthworms. This effect is likely due to the increasing concentration of phytochemical components in the extract, attributed to various secondary metabolites in the extract, including saponins, tannins, alkaloids, amino acids, non-protein compounds, and glycosides (Guarrera, 1999). These compounds are known for their free radical scavenging activities, which contribute to their pharmacological effects (Talari *et al*., 2017). Additionally, *A. digitata* has been traditionally used for treating diarrhea and dysentery, which might also be linked to its anthelmintic properties, given that these symptoms are often associated with parasitic infections. Previous research has confirmed the strong anthelmintic activity of both methanol and ethanol extracts of *A. digitata* leaf. Notably, the ethanolic extract demonstrated superior anthelmintic efficacy compared to the methanolic fractions under similar conditions.

Conclusion: The ethanolic and methanolic extracts of *A. digitata* stem bark showed promising anthelmintic activity most especially after 60 minutes of exposure, with effectiveness increasing in a dose-dependent manner. Interestingly, the observed activity of these extracts was comparable to that of the synthetic anthelmintic drug albendazole. We suggest that further studies be conducted to determine the phytochemical, elemental and antioxidant activities of the plant extract as well as determine the active fraction of the plants elucidating the anthelmintic activity.

*Declaration of Conflict of Interest***:** The authors declare no conflict of interest

*Data Availability Statement***:** Data are available upon request from the first author or corresponding author or any of the other authors

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